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Degradation of almond pellicle color coordinates at different storage temperatures

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Abstract

The pellicle or seed coat of almond kernels is subject to darkening during long-term storage and may affect the marketability of the stored product. Environmental conditions during storage and genetic factors both affect the extent of darkening during the storage period. The degree of pellicle color change of five distinct almond accessions was examined during long-term storage at 2, 22 and 32 °C. Pellicle luminosity, chroma and hue angle were measured on 12 dates throughout an 11-month storage period. An ANCOVA was used, with storage temperature being a covariate, to examine the relative differences in luminosity, chroma and hue angle during the storage progression. A comparison of weighted simple linear regression equations was used to distinguish between different rates of pellicle color coordinate degradation during the storage period. When averaged across the three storage temperatures, almond accession Padre consistently had significantly lower pellicle luminosity and chroma values throughout the storage period as compared with the other four almond accessions ($p \le 0.05$). While pellicle hue angle values of Padre were significantly lower than those of Nonpareil at the start of the test ($p \le 0.05$), Nonpareil's pellicle hue angles were significantly lower than those of Padre at the end of the storage period ($p \le 0.05$). Almond accession Nonpareil had the largest percentage decrease of the five almond accessions for pellicle luminosity (36.9%) and hue angle (12.5%). Regression analysis revealed significant differences in degradation rates of pellicle luminosity and chroma at all three storage temperatures ($p \le 0.05$), but significant differences in pellicle hue angle degradation were only evident at the lowest storage temperature ($p \le 0.05$).
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Keywords: Chroma; Genetic variability; Hue angle; Luminosity; Oxidative rancidity

1. Introduction

California's extensive almond acreage has consistently provided 70% of the world's annual crop for the last several decades. Acreage continues to grow, and a third consecutive 450+ million kg harvest year has been realized (Anon., 2005). Marketing this tremendous yield occurs year-round and requires that large volumes of almonds be stored for many months after the annual harvest.

Storage conditions vary with almond processor and with the anticipated time in storage. A controlled low oxygen atmosphere in the storage space has been shown to be superior to normal atmospheric conditions relative to almond flavor retention, and was independent of storage temperature (Guadagni et al., 1978). Under normal atmospheric conditions, cold (0–5 °C) and humid (65% RH) conditions are optimal for long-term storage of shelled almonds (Anon., 2003; Perry and Sibbett, 1998) but these conditions are expensive to maintain for large volumes of stored products. Besides storage temperature and relative humidity, factors such as kernel moisture content, presence/absence of light, oxygen level, and the form of stored nut (in-shell, shelled, blanched, roasted, diced, paste, etc.) can affect the quality of almonds coming out of storage (Baiano and Del Nobile, 2005; Kazantzis et al., 2003; Pearson, 1999; Burg, 1998). Recommendations on specific shelf life vary between researchers, but there is general agreement that in-shell almonds can be

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stored for at least a year under ambient conditions without a loss of kernel quality.

Freshly shelled almond kernels exhibit varietal pellicle color differences which are under genetic control (Kester and Asay, 1975). Although color is among the most important attributes consumers use to distinguish product quality (Clydesdale, 1991), published USDA standards for grades of shelled almonds do not specify kernel color as a means for distinguishing varietal character (Anon., 1997). However, consumer preferences play a role in determining marketability of agricultural products (Bonner and Nelson, 1985), and almonds that are uncharacteristically dark can be perceived as having turned rancid, or simply having lost freshness. Oxidative rancidity is the result of a reaction between the kernel's unsaturated fatty acids and oxygen. In almonds, this reaction is reduced or delayed by the presence of antioxidative phenolic compounds such as α-tocopherol and other free radical scavengers, which have recently been identified in the almond pellicle (Sang et al., 2002).

Almond breeding efforts at the USDA Agricultural Research Service's Parlier, CA location are focused on developing new varieties with high quality kernels that are pollen intercompatible and visually non-distinguishable from the commercially important Nonpareil cultivar (Ledbetter and Palmquist, 2002). Annual kernel evaluations of cultivars and experimental almond accessions include both objective and subjective measures, including an analysis of color coordinates for each accession's pellicle. In 2003 we observed that short-term storage of shelled almond accessions under ambient environmental conditions led to visually distinct changes in pellicle color indices as compared to their original values. These observations led to the current study, where selected almond accessions were evaluated for changes in color indices under three storage temperature regimes during 11 months of storage.

2. Materials and methods

Almonds utilized in this study were grown, harvested and hulled/shelled at the Agricultural Research Service's San Joaquin Valley Agricultural Sciences Center in Parlier, CA. The five almond accessions used (Nonpareil, A25-40, 82-73, K3-90 & Padre) were grown in a single orchard under the same regime of cultural conditions. All trees were grown on 'Nemaguard' rootstock and were in their ninth leaf during the study's first year (2003).

Nonpareil and experimental accessions A25-40, 82-73 and K3-90 are soft-shelled and early ripening. The experimental accessions are all very similar to Nonpareil in kernel size and shape. Nonpareil's kernel pellicle pubescence is rated as low (Gülcan, 1985), and pubescence on the three experimental accessions' kernels would be described as the same. Cultivar Padre is a hard-shelled roasting almond that differs from the others in kernel size, shape and color. Padre's pellicle is more noticeably wrinkled than that of the other four accessions, and this difference enhances its suitability as a roasting almond. Pellicle pubescence of Padre would be described as intermediate, slightly more than that of the other four studied accessions. Padre harvests approximately 20 days after the other four accessions. After each almond accession was hulled/shelled in the field, almond kernels were brought to the lab and held in cold storage (2 °C) until all accessions were ready for evaluation. Kernel pellicle color coordinates were measured with a Minolta Chroma Meter CR-200 (Minolta Camera Co. Ltd., Osaka 451, Japan) equipped with an 8 mm measurement aperture. The measuring head of the CR-200 employs diffuse illumination of a xenon arc lamp pulse within a mixing chamber to obtain color coordinate values from non-uniform sample surfaces. The pellicle luminosity, chroma and hue angle were recorded for kernel samples of each almond accession.

In the initial pellicle color analysis performed on the 2003 crop, sample size was 25 kernels per almond accession. Kernels used for the pellicle color analysis were examined carefully to exclude scratched or damaged kernels that might alter color coordinate readings. After the initial evaluation of pellicle color, kernel samples were bagged in plastic, and then in kraft paper bags before storage. Samples were stored at 22 °C for 120 days prior to a re-analysis of color coordinates of the original almond kernel samples.

A more comprehensive analysis of pellicle color change was again performed on almonds grown in 2004. Kernels from each of the five almond accessions were selected carefully after shelling for color uniformity within each accession, and for unchipped/unscratched kernels. Selected almond samples were first placed in plastic bags, and then in kraft paper bags to exclude light and stored at 2, 22 and at 32 °C. Thirty kernels were evaluated per accession and temperature treatment. Samples were analyzed for luminosity, chroma and hue angle on 12 successive and random dates throughout the next 11 months.

2.1. Statistical analyses

For the initial pellicle color change analysis conducted in 2003, a completely random design (CRD) was used to examine color coordinate changes in five almond accessions initially after cracking and again after a 4-month storage period at 22 °C. The factors in ANOVAs for dependent variables luminosity, chroma and hue angle were 'almond accession' as a fixed effect, consisting of Nonpareil, A24-40, 82-73, K3-90 and Padre; and 'storage' as a fixed effect with initial and 4-month storage values. For the comprehensive analysis of pellicle color change in 2004, a CRD was used to examine light characteristics of the five almond varieties as a function of days after cracking that were stored at three temperatures (2, 22 and at 32 °C). Analyses of covariance (ANCOVAs) were used to analyze almond accession differences for luminosity, chroma, and hue angle for each level of days after cracking using storage temperature as a covariate. Levene's homogeneity of variance test was performed to check for data transformation necessity. If a significant F-test statistic was obtained in the analyses at $p \leq 0.05$, a Duncan's new multiple range test at the 0.05 level was used as the multiple comparison procedure for determining differences between the almond accessions. PROC REG and PROC GLM were the statistical procedures used for these analyses (SAS PC Windows Version 9.1, SAS Institute, Inc., Cary, NC, USA).

Weighted simple linear regression equations of dependent variables luminosity, chroma, and hue angle as a function of days after cracking were calculated for each of five almond accessions stored at three different temperatures. Weighted regression was used to compensate for variance heterogeneity of the replications.

Confidence intervals (95%) on weighted regression slopes for luminosity, chroma, and hue angle equations were used to analyze almond accession differences at each level of storage temperature for simple linear regressions. PROC REG was the statistical procedure used for weighted regression analyses (SAS PC Windows Version 9.1, SAS Institute, Inc., Cary, NC, USA).

3. Results and discussion

Almond samples examined during the initial storage trial of 2003 revealed large differences in pellicle color coordinate changes between the five accessions after 4 months of storage at 22 °C (Table 1). The main effects almond accession and storage, as well as their interaction were observed to be significant ($p \leq 0.05$) in affecting luminosity, chroma and hue angle values during storage (data not presented). Of the five almond accessions, Nonpareil decreased most during storage in both pellicle luminosity and chroma (22.5% and 19.4%, respectively), and these decreases were significant ($p \leq 0.05$). A25-40 and 82-73 also exhibited significant ($p \leq 0.05$) decreases in luminosity during storage while K3-90 did not differ significantly ($p \leq 0.05$) in pellicle luminosity value between the initial evaluation and at the end of the 4-month storage. Pellicle luminosity of Padre increased significantly ($p \leq 0.05$) during the storage period. Pellicle chroma values decreased significantly ($p \leq 0.05$) during storage for almond accessions Nonpareil, A25-40 and 82-73, while no significant differences were noted between initial pellicle chroma values and after 4 months of storage for accessions K3-90 and Padre. Pellicle hue angle decreases during storage were most pronounced (8.3%) in A25-40, and significant ($p \leq 0.05$) hue angle decreases were observed for all almond accessions.

Table 1
Mean values of main effects 'almond accession' and 'storage' and their associated interaction for luminosity, chroma and hue angle pellicle values from five almond accessions stored 4 months at 22 °C

	Luminosity	Chroma	Hue angle
Main effect			
Almond accession			
Nonpareil	44.7	34.8	67.2
A25-40	49.4	31.1	70.9
82-73	43.3	31.4	68.4
K3-90	37.5	29.9	65.1
Padre	35.2	26.1	63.0
Storage			
Initial values	43.8	32.1	69.0
After 4 months	40.3	29.3	64.8
Interaction effect			
Nonpareil initial	50.4 b[a]	38.5 a	69.5 c
Nonpareil stored	39.1 e	31.0 c	64.9 f
A25-40 initial	53.5 a	32.8 b	73.9 a
A25-40 stored	45.4 c	29.4 d	67.8 d
82-73 initial	44.3 c	32.5 b	70.9 b
82-73 stored	42.4 d	30.3 cd	66.0 ef
K3-90 initial	37.4 f	30.2 cd	66.5 e
K3-90 stored	37.5 f	29.7 d	63.7 g
Padre initial	33.3 g	26.2 e	64.1 fg
Padre stored	37.0 f	25.9 e	61.9 h

[a] Means followed by the same letter within a specific dependent variable are not significantly different at the 0.05 level according to a Duncan's multiple range test.

After the 2004 harvest, almond samples of the five accessions were again analyzed for initial pellicle color coordinates prior to storage under three temperature regimes (2, 22 and 32 °C). Storage temperature was treated as a covariate throughout this study, and was observed to be a highly significant ($p \leq 0.01$) factor in pellicle luminosity, chroma and hue angle at all sampling dates except during the initial period prior to storage (data not presented). Pellicle luminosity, chroma and hue angle for the five analyzed almond accessions are presented for each of the 12 sampling dates, averaged across the three storage temperatures (Table 2).

While pellicle luminosity, chroma and hue angle values decreased for each of the five almond accessions throughout the 11-month storage period, changes in the color coordinates were not uniform for each of the five almond accessions. Accession A25-40 retained significantly higher pellicle luminosity values than the other four almond accessions ($p \leq 0.05$) throughout the length of storage. Throughout the storage period, accession 82-73 always had significantly lower pellicle luminosity than A25-40 ($p \leq 0.05$) and significantly higher luminosity than Nonpareil ($p \leq 0.05$). Accessions K3-90 and Nonpareil began the storage period with Nonpareil exhibiting significantly higher pellicle luminosity than K3-90 ($p \leq 0.05$). By the end of the storage period the situation was reversed with K3-90 having significantly higher luminosity than Nonpareil ($p \leq 0.05$). Padre's pellicle luminosity remained significantly lower than the other four accessions throughout the study ($p \leq 0.05$).

Pellicle chroma values for Padre were also significantly lower than those of the other four almond accessions ($p \leq 0.05$) throughout the storage period. At the onset of the test, pellicle chroma of 82-73 was significantly higher than that of the other almond accessions ($p \leq 0.05$). By day 28, the first sampling date during storage, there were no significant differences in pellicle chroma for 82-73 and K3-90, and these two accession's pellicle chroma values were significantly higher than that of the other three accessions ($p \leq 0.05$). This

Table 2
Pellicle luminosity, chroma and hue angle average values of five almond accessions computed across three storage temperatures during 323 days of storage

Days after initial	Accession	Luminosity	Chroma	Hue angle
Initial	Nonpareil	48.3 c[a]	33.3 c	67.2 d
	A25-40	58.6 a	33.3 c	76.1 a
	82-73	49.9 b	36.9 a	71.0 b
	K3-90	47.1 d	36.2 b	68.8 c
	Padre	40.6 e	27.4 d	66.1 e
28	Nonpareil	45.0 c	31.3 b	66.2 c
	A25-40	54.7 a	30.8 b	73.5 a
	82-73	46.2 b	33.9 a	68.8 b
	K3-90	44.2 c	34.1 a	67.6 c
	Padre	37.5 d	25.7 c	64.8 c
56	Nonpareil	42.1 c	30.3 b	64.5 d
	A25-40	51.3 a	30.4 b	71.4 a
	82-73	43.7 b	33.1 a	67.6 b
	K3-90	41.7 c	33.5 a	66.0 c
	Padre	35.3 d	25.6 c	62.9 e
84	Nonpareil	39.3 c	29.9 b	64.2 c
	A25-40	50.3 a	29.9 b	69.8 a
	82-73	42.6 b	32.6 a	66.5 b
	K3-90	39.1 c	31.8 a	64.7 c
	Padre	33.3 d	23.7 c	62.2 d
110	Nonpareil	39.1 c	29.2 b	64.1 d
	A25-40	47.7 a	28.7 b	69.8 a
	82-73	40.4 b	31.3 a	66.4 b
	K3-90	37.2 c	31.7 a	65.2 c
	Padre	33.4 d	23.8 c	62.7 e
141	Nonpareil	36.7 b	28.9 b	63.5 c
	A25-40	47.5 a	27.7 c	69.2 a
	82-73	39.3 b	30.3 a	66.2 b
	K3-90	37.2 c	30.8 a	65.1 b
	Padre	31.9 d	22.9 d	62.7 c
174	Nonpareil	37.0 b	27.3 c	64.1 c
	A25-40	44.4 a	26.6 c	68.6 a
	82-73	36.9 b	29.8 b	65.3 b
	K3-90	37.5 b	31.3 a	65.5 b
	Padre	31.9 c	23.8 d	62.7 d
208	Nonpareil	36.9 c	28.5 b	63.9 c
	A25-40	45.1 a	27.5 c	68.9 a
	82-73	38.0 b	30.3 a	66.1 b
	K3-90	36.1 c	30.7 a	65.4 b
	Padre	31.2 d	23.4 d	62.7 d
230	Nonpareil	36.6 bc	28.2 b	63.7 c
	A25-40	45.0 a	27.6 b	68.7 a
	82-73	37.5 b	30.1 a	65.6 b
	K3-90	35.7 c	30.4 a	64.5 c
	Padre	30.4 d	23.3 c	61.9 d
260	Nonpareil	35.2 b	27.8 b	62.3 d
	A25-40	43.0 a	27.8 b	67.8 a
	82-73	35.8 b	29.5 a	64.7 b
	K3-90	34.2 c	30.0 a	63.6 c
	Padre	29.2 d	22.9 c	61.5 d
294	Nonpareil	31.6 c	27.4 b	59.3 d
	A25-40	42.1 a	27.7 b	66.6 a
	82-73	35.8 b	29.5 a	64.7 b
	K3-90	34.2 c	30.0 a	63.6 c
	Padre	29.1 d	22.9 c	60.2 cd
323	Nonpareil	30.5 d	27.2 b	59.1 d
	A25-40	42.0 a	27.4 b	66.6 a
	82-73	35.4 b	29.4 a	64.0 b
	K3-90	34.3 c	28.8 a	60.5 c
	Padre	28.7 e	22.6 c	60.3 c

[a] Average values of luminosity, chroma, and hue angle for each 'days after initial' category followed by the same letter are not significantly different based on Duncan's multiple range test at the $p = 0.05$ level.

situation remained relatively constant throughout the remainder of the test with 82-73 and K3-90 both having significantly higher pellicle chroma values than Nonpareil, A25-40 and Padre at the end of the storage period ($p \leq 0.05$). Padre's pellicle chroma values were always significantly lower than those of the other four accessions at each sampling date during the storage period ($p \leq 0.05$).

Nonpareil's pellicle hue angle values were initially significantly less than those of A25-40, 82-73 and K3-90 ($p \leq 0.05$), and initially significantly higher than those of Padre ($p \leq 0.05$). At various dates during the storage period Nonpareil and Padre did not differ significantly in pellicle hue angle, but by the end of the storage period, Padre's pellicle hue angle was significantly higher ($p \leq 0.05$) than that of Nonpareil. Vacillation in statistical ranking of pellicle hue angle values also occurred during the storage period for 82-73 and K3-90. But in this case 82-73's pellicle hue angle values were initially significantly higher than those of K3-90 ($p \leq 0.05$), and were also significantly higher than K3-90 at the end of the storage period ($p \leq 0.05$). Pellicle hue angle of A25-40 was significantly higher than that of the other four almond accessions throughout the storage period ($p \leq 0.05$).

Weighted regression analyses were used in 2004 to compare degradation rates of pellicle color coordinates for the five almond accessions. Best-fit simple linear weighted regressions took the forms:

- luminosity $= \beta_0 + \beta_1\sqrt{\text{(days after cracking)}}$
- chroma $= \beta_0 + \beta_1 \ln(\text{DAC})^2$
- hue angle $= \beta_0 + \beta_1\sqrt{\text{DAC}}$

With these equations, direct comparisons of the β_1 slopes of the five almond accessions within a single storage temperature could be evaluated. Table 3 presents slope estimates and associated R^2 values for pellicle luminosity, chroma and hue angle degradation of the five almond accessions during 11 months of storage at each of three storage temperatures. For each of the color coordinates, degradation rates were observed to be increasingly more negative as storage temperature increased in all cases except for almond accession A25-40 for color coordinate chroma. At the 2 °C storage temperature, almond accession Padre had a significantly ($p \leq 0.05$) lower slope than Nonpareil, A25-40 and 82-73 for pellicle luminosity, and differed significantly ($p \leq 0.05$) from only A25-40 for both chroma and hue angle. Except for this significant difference between Padre and A25-40's

Table 3
β_1 slope estimates and fit statistics (R^2) from weighted linear regressions of pellicle luminosity, chroma and hue angle for five almond accessions stored 11 months under three temperatures

Almond accession	Storage temperature (°C)		
	2	22	32
Luminosity[a]			
Nonpareil	−0.849 a[b] (0.93)	−0.955 ab (0.94)	−1.0745 ab (0.92)
A25-40	−0.843 a (0.95)	−1.069 a (0.96)	−1.093 a (0.98)
82-73	−0.775 a (0.95)	−0.879 abc (0.95)	−1.020 a (0.98)
K3-90	−0.655 ab (0.95)	−0.747 bc (0.93)	−0.932 ab (0.97)
Padre	−0.566 b (0.96)	−0.695 c (0.98)	−0.813 b (0.98)
Chroma[a]			
Nonpareil	−0.144 ab (0.86)	−0.191 ab (0.92)	−0.203 ab (0.91)
A25-40	−0.191 a (0.87)	−0.193 ab (0.81)	−0.167 ab (0.73)
82-73	−0.188 ab (0.84)	−0.227 a (0.92)	−0.263 a (0.97)
K3-90	−0.161 ab (0.93)	−0.220 a (0.98)	−0.253 ab (0.85)
Padre	−0.093 b (0.66)	−0.131 b (0.76)	−0.189 b (0.92)
Hue angle[a]			
Nonpareil	−0.230 ab (0.56)	−0.389 a (0.76)	−0.593 a (0.69)
A25-40	−0.306 a (0.84)	−0.511 a (0.86)	−0.629 a (0.89)
82-73	−0.165 ab (0.75)	−0.335 a (0.83)	−0.605 a (0.89)
K3-90	−0.130 ab (0.36)	−0.407 a (0.78)	−0.537 a (0.72)
Padre	−0.103 b (0.47)	−0.299 a (0.87)	−0.522 a (0.89)

[a] Color coordinate.
[b] β_1 slope estimates having the same letter within a color coordinate grouping and specific storage temperature do not differ significantly when 95% confidence intervals overlap.

rate of pellicle hue angle degradation, no other significant ($p \leq 0.05$) differences were observed for hue angle degradation rates between the other almond accessions at either of the higher storage temperatures. At 22 °C, luminosity value slope was significantly ($p \leq 0.05$) higher for A25-40 than either K3-90 or Padre. Padre's luminosity value degradation was also significantly ($p \leq 0.05$) lower than that of Nonpareil at the 22 °C storage temperature. Pellicle chroma degradation rates of 82-73 and K3-90 were significantly ($p \leq 0.05$) higher than that of Padre at 22 °C, and did not differ significantly from those of either Nonpareil or A25-40. At the highest storage temperature, Padre's rate of pellicle color degradation was significantly ($p \leq 0.05$) less than that of 82-73 for chroma values, and significantly ($p \leq 0.05$) less than that of both 82-73 and A25-40 for luminosity values. At this high storage temperature, Nonpareil and K3-90 did not differ significantly from A25-40, 82-73 or Padre for rate of pellicle luminosity degradation. Pellicle chroma degradation rate of Nonpareil, A25-40 and K3-90 at the highest storage temperature did not differ significantly from that of either 82-73 or Padre.

Direct comparisons between experiments conducted in 2003 and 2004 are not obtainable due to differences in the performed analyses. However, there are some similarities in color coordinate degeneration that can be noted with respect to the five almond accessions. Of particular note are Nonpareil's pellicle luminosity values and the percentage change recorded in the 2 years of the study. As was similarly observed during 2003, Nonpareil's pellicle luminosity decreases were the highest among the five almond accessions as measured on a percentage basis (36.9%) across the three storage temperatures. Percentage decreases in pellicle hue angle values during 2004 were also numerically highest for A25-40 (12.5%) as compared with the other four accessions. This same accession was observed to have the largest decrease in hue angle after 4 months storage at 22 °C in 2003.

Perceived visual differences were evident and distinct at the end of the storage period when comparing each of the five almond accessions' samples stored at the different temperatures. Samples of all almond accessions stored at 2 °C appeared more attractive, and most similar to a freshly shelled product. After 11 months storage at 22 °C kernels of Nonpareil no longer had a fresh or 'new crop' appearance. Nonpareil's pellicles were darker and with a visually cinnamon-like appearance that would probably lead an observant consumer to question product freshness. The cinnamon-like appearance increased in intensity for Nonpareil kernels stored at 32 °C, and the sample was reminiscent of kernels that had been roasted. Samples of both K3-90 and 82-73 had a similar roasted appearance in the 32 °C storage samples, though not as visually distinct as that of Nonpareil. Perceived visual differences were much less for accessions A25-40 and Padre, compared across the temperature treatments at the end of the storage period. The noted differences in pellicle color degradation might be associated with similar changes in antioxidant levels present in the almond pellicles. The almond pellicle is known to be a rich source of antioxidants with 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (Sang et al., 2002). Other researchers have shown decreases in levels of α-tocopherol and associated increased accumulations of hydroperoxides and malondialdehyde in aged almond seed, suggesting fatty acid peroxidation associated with seed deterioration during storage (Zacheo et al., 2000). With the year-round marketing of stored almonds and sometimes sub-optimal storage conditions, it would be a benefit to identify particular almond accessions more tolerant of degradative changes associated with long-term storage of almonds. In the present study significant differences were demonstrated between almond accessions for rate of pellicle color degradation during storage. Subsequent studies might follow with these same almond accessions to determine whether differences in associated biochemical changes exist that correlate with the observed changes of color coordinates.

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